PROTECTIVE EFFECT OF SYSTEMIC ADMINISTRATION OF PRAVASTATIN AGAINST NOISE-INDUCED HEARING LOSS IN THE FISCHER 344/NHsd RAT SUBSTRAIN

Capstone Project

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By

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ABSTRACT

The deleterious effects of noise-induced hearing loss (NIHL) on the auditory system have been well documented, contributing to temporary thresholds shifts (TTS) and permanent threshold shifts (PTS). The generation of reactive oxygen species (ROS) greatly contributes to the pathogenesis of NIHL, and as such is a target for pharmacological intervention by strengthening the antioxidant defense system in the body.

Previous studies suggest that pravastatin may lower ROS production and block apoptotic cell death. As such, the aim of this study was to examine the protective effect of pravastatin against noise in the Fischer 344/NHsd rat substrain. The noise condition was a 2-octave band continuous noise of 4 kHz – 16 kHz, delivered at 110 dB SPL combined with 120 dB pSPL impacts. In the treated group, pravastatin was administered via intraperitoneal injections (12 mg/kg) 24 hours before noise exposure; 1 hour prior to and 1 hour following noise exposure; and then 24 hours post noise exposure.

Threshold shifts for the treated versus untreated groups were assessed at 6 frequencies (5, 10, 15, 20, 30, and 40 kHz) and were obtained 1 day and 14 days after the noise to document TTS and 28 days post noise to document PTS. The 3-way ANOVA did not show a significant main effect ($p < .05$) of Group or Frequency, nor any significant interaction involving treatment groups. There was a trend toward significance for the interaction of Day and Group ($p = 0.057$). Recovery functions indicated that, from Day 14 to Day 28, the treated group demonstrated a decrease in thresholds that the untreated control group did not. While this study did not indicate a significant protective

effect of pravastatin, further investigation of pravastatin's protective capacity against NIHL is needed to extrapolate therapeutic strategies caused by ROS overproduction.

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LIST OF ABBREVIATIONS

4-HNE	4-hydroxynonenal
ABR	auditory brainstem response(s)
ALCAR	acety-L-carnitine
ANOVA	analysis of variance
ARHL	age-related hearing loss
CANS	central auditory nervous system
cm	centimeter
dB	decibel(s)
dB(A)	A-weighting
dB pSPL	peak sound pressure level
dB SPL	sound pressure level
DNA	deoxyribonucleic acid
EAM	external auditory meatus
EP	endocochlear potential
FDA	Food and Drug Administration
HC	hair cell(s)
HL	hearing loss
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
Hz	hertz
IE	inner ear
IHC	inner hair cell(s)
K+	potassium

kg	kilogram
kHz	kilohertz
L	liter
LDL	low-density lipoprotein
Leq	equivalent continuous noise level
LNAC	n-l-acetyl cysteine
ME	middle ear
mg	milligram
min	minute
ml	milliliter
ms	millisecond
NADPH	nicotinamide adenine dinucleotide phosphate
NIHL	noise-induced hearing loss
O_2	oxygen
OE	outer ear
OHC	outer hair cell(s)
PTS	permanent threshold shift
ROS	reactive oxygen species
sec	second
SNR	signal-to-noise ratio
TM	tympanic membrane
TTS	temporary threshold shift

CHAPTER 1

Introduction

The adverse effects of ambient noise and chronic noise exposure on the auditory system have been well documented. One-third of persons with hearing impairment partially attribute their loss to noise exposure, mainly occupational noise exposure (NIH, 1990). In an industrial environment, exposure to 85 – 90 dB(A) noise levels can lead to hearing loss (HL). Additionally, noise exposure may adversely affect one's health and quality of life, which implies that noise exposure is a significant public health concern in industrialized nations (Passchier-Vermeer & Passchier, 2000). In addition to its effects on hearing, noise may be perceived as an environmental stressor that impacts one's general health and cognitive processes (Stansfeld & Matheson, 2003). Literature regarding the non-auditory effects of noise centers on the psychological and physiological effects of noise exposure, namely communication interference, sleep interference, cognitive performance, and cardiovascular and neuroendocrine factors.

1.1. Cochlear mechanics

In a typically developing person, the peripheral auditory system consists of an outer ear (OE), middle ear (ME), and an inner ear (IE). Each portion influences transmission of acoustic stimuli. The process of sound transmission is initiated when an acoustic stimulus enters the auditory system via the OE, which is comprised of the pinna and the external auditory meatus (EAM). The acoustic stimulus then traverses through

the EAM to the ME; the EAM directs acoustic signals to the tympanic membrane. The resonance properties of the EAM lead to amplification of sounds in the 1.5 kHz – 7 kHz range. The ME space, or tympanic cavity, consists of the tympanic membrane, ossicular chain (i.e., malleus, incus, and stapes), and the Eustachian tube. Additionally, structures housed within the tympanic cavity consist of tendons from 2 ME muscles (i.e., tensor tympani and stapedius). The primary function of the ME is to serve as an impedance matching element between the air-filled ME and the fluid-filled IE. After passing through the OE and ME, the acoustic stimulus is delivered to the cochlea. As part of the sound transmission process, the OE and ME are responsible for conducting sound energy and increasing sound intensity. The acoustic stimulus is converted from acoustic energy to electrochemical energy at the level of the cochlea. It is then the responsibility of the auditory nerve to transmit the electrochemical impulses centrally.

The cochlea has several thousand hair cells (HC) and nerve endings (afferent and efferent nerve fibers). The organ of Corti is the sensory organ in the IE that contains the 2 types of HC: 1) outer HC (OHC) and 2) inner HC (IHC) (Musiek & Baran, 2007b). Both types of HC form synapses with the nerve fibers, which allows communication with the nerve fibers. The majority of efferent nerve fibers innervate OHC. In contrast, the majority (~85 – 95%) of afferent nerve fibers innervate IHC. On the apical end of each HC are stereocilia, which respond to fluid motion from an acoustic stimulus (Musiek & Baran, 2007a). The deflection of stereocilia leads to an action potential in a process called depolarization. Conversely, hyperpolarization inhibits the onset of an action potential. Both depolarization and hyperpolarization must occur for the active mechanism to transduce sound effectively. The cochlea is organized to enable HC transduction. Insult to

the cochlea has the potential to cause the stereocilia to become flaccid or fuse the stereocilia together, thus not permitting the depolarization process to occur (Musiek & Baran, 2007a). The HC transduction process commences when an acoustic stimulus evokes vibrations of the cochlear partition that are then converted into electrical activity. On OHC and IHC, the mechanically gated transduction channels are located near the tip of the stereocilia; the transduction channels are responsible for converting sound into neural activity. The stereocilia deflect toward the tallest stereocilia, thereby increasing the number of open transduction channels, resulting in the depolarization process (Musiek & Baran, 2007a). Conversely, deflection towards the shortest stereocilia increases the number of closed transduction channels, resulting in hyperpolarization of a given HC. When channels are open, positive potassium (K+) ions in the endolymph flow into the HC. The active HC transduction mechanism facilitates communication between the HC and the nerve fibers (Musiek & Baran, 2007a). An IHC action potential leads to release of excitatory neurotransmitter into the synaptic cleft with the afferent auditory nerve fibers terminals. The afferent auditory pathway (IHC transduction) transmits information to the central auditory nervous system (CANS). The efferent auditory pathway, or descending pathway, transmits information from the central levels of the auditory system to the peripheral levels of the auditory system. Both afferent and efferent pathways must be functioning in order for a person to optimally perceive sounds in the variety of listening environments.

1.2. Pathogenesis of NIHL

Noise-induced cell death may occur by various complex mechanisms. As a result of acoustic trauma exposures, active mechanisms at the cellular level trigger HC death.

Noise exposure increases levels of ROS during and after the noise exposure (Henderson, Bielefeld, Harris, & Hu, 2006). ROS include oxygen-based free radicals, chemical species with an unpaired electron, thus capable of altering electron arrangements in stable molecules. ROS break down cell membranes through lipid peroxidation, leading to cell death. The expression 'oxidative stress' describes numerous detrimental processes due to an imbalance between excessive formation of ROS and the significant decrease in the effectiveness of antioxidant defenses. In addition, genetic factors and aging may cause an increased concentration of ROS. A buildup of ROS in cells will cause damage to nucleic acids, lipid membranes, and proteins, thereby disrupting normal cellular functions.

The biological processes of cell death after noise exposure include necrotic and apoptotic cell death. Necrosis is acquired cell death, or a passive form of cell death, that is due to physical or chemical insults. Cell swelling results; in so doing, this leads to rupture of the cell and a subsequent releasing of its contents into the extracellular spaces. Because the cell disassembles, the ability to regulate the intracellular environment has been lost. This process evokes an inflammatory response and causes irreversible damage to surrounding tissue.

Necrosis was thought to be the primary mode of cell death in the noise-exposed cochlea. However, the existence of apoptosis, or programmed cell death, has also been observed in the noise-damaged cochlea (Hu, Guo, Wang, Henderson, & Jiang, 2000; Hu, Henderson, & Nicotera, 2002). Apoptosis is a necessary part of nervous system development in all animals. Unlike cell death due to injury, apoptotic cell death is required for proper development and morphogenesis (Henderson et al., 2006). Furthermore, programmed cell death is needed to destroy cells that represent a threat to

the integrity of multicellular organisms, such cells with viral infection, cells in states of extreme stress, cells with DNA damage, and cancer cells. Apoptosis is a regulated process, conducted in a predictable and reproducible pattern, which requires energy expenditure (Henderson et al., 2006). The cell membrane remains intact, and the cell pulls away from bordering cells. Morphologic cellular changes include the following: blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and DNA fragmentation. During apoptosis, inflammation does not occur since cellular contents are not released. The noise-induced lesion of dead OHC results from a combination of apoptosis and necrosis. However, the OHC lesion grows primarily via apoptosis, and the lesion grows toward the basal side of the cochlea (Hu et al., 2002).

Several different forms of cellular damage occur, because noise abuses the cochlea metabolically and mechanically. Also, HC dying for several days or weeks after an exposure presents two questions: 1) Can HC be protected, thus preventing subsequent cochlear damage and HL, by neutralizing ROS as they are formed? 2) Can researchers limit HC loss via pharmacological intervention that inhibits the disassembly of the HC through apoptosis?

1.3. Current intervention strategies

Noise-induced HL (NIHL), due to acute or chronic noise trauma, depends on the frequency content, sound pressure level, exposure time, in addition to an individual's susceptibility to noise. NIHL may cause substantial communication difficulties, which can be partially managed by the utilization of hearing aids, assistive listening technology, and aural rehabilitative measures. In general, HL is currently managed by device-related modalities, which may generate reasonable results for some individuals. These

rehabilitation strategies may improve the quality of life for individuals with NIHL; however, it is important to note that technology does not restore natural hearing nor does it replace the processing capabilities of a normal auditory system.

HC regeneration, currently, is not an option for reversing the effects of NIHL. Also, there are no drugs that have been approved by the Food and Drug Administration (FDA) for the prevention or treatment of NIHL. Consequently, there is a need for researchers to uncover alternative therapies to protect against or lessen the effects of NIHL. There is a search for otoprotective agents or pharmacological interventions that could potentially protect cochlear HC from excessive noise exposure, or lessen its harmful effects. Augmentation of the antioxidant defense system is an objective for pharmacological prevention. In terms of intervention strategies, there are 2 schools of thought, one being 'protection' (i.e., providing the drug treatment before the noise exposure) and the other being 'rescue' (i.e., providing the drug treatment after the noise exposure). It is important to note that though some otoprotective agents have shown protection against NIHL in animal studies, the agents may pose safety issues for humans if administered systematically.

As previously discussed, an increase in ROS, byproducts of mitochondrial respiration, plays a significant role in the exacerbation of NIHL. Therefore, it is thought that increasing antioxidant activity may counteract ROS formation, or apoptotic intracellular signaling process may be impeded in order to promote cell survival. There is evidence that antioxidants may be beneficial when treating or preventing the onset or progression of NIHL. Antioxidant vitamins scavenge free radicals. Additionally, they prevent oxidative damage from spreading by interrupting lipid peroxidation. Many

otoprotective agents are micronutrients that occur in one's typical daily diet, such as vitamins A, C, and E, and magnesium (Le Prell, Hughes, & Miller, 2007). The mineral magnesium activates enzymes, contributes to protein synthesis and energy production, and assists in regulating calcium levels, when in combination with copper, zinc, potassium, and vitamin D. Magnesium preserves blood flow to the IE and aids in healing the cells. Furthermore, resveratrol, a nutritional supplement found in the skin of red grapes and an active component in red wine, may provide some protection against NIHL. A study by Seidman, Babu, Tang, Naem, and Quirk (2003) demonstrates a protective effect of resveratrol on NIHL, in which the resveratrol group showed reduced threshold shifts after a 7 week resveratrol treatment.

Acety-L-carnitine (ALCAR) and n-l-acetyl cysteine (LNAC) have also been shown to play a significant role in ameliorating the effects of NIHL. ALCAR is a molecule that aids in maintaining mitochondrial efficiency. LNAC promotes cell detoxification and increases levels of the antioxidant glutathione, which neutralizes the toxic hydroxyl radical and peroxynitrite. D-Methionine may also increase glutathione levels within the cell. Both ALCAR and LNAC are antioxidants that may protect the cochlea from impulse noise (Kopke et al., 2005). To illustrate, in a study by Kopke et al. (2005), chinchillas received ALCAR or LNAC as a pre- and post-treatment to lessen the effects of impulse noise. Results revealed that PTS were roughly 10 – 30 dB less than the control group 3 weeks after noise exposure.

1.4. Pravastatin

As reviewed above, numerous pharmacological strategies to attenuate NIHL have been tested in animal models. Acoustic trauma generates ROS in the pathogenesis of

NIHL, and as such is a focus for pharmacological intervention by strengthening the antioxidant defense system in the body. HMG-CoA reductase inhibitors (statins) are a class of prescription drugs to reduce blood levels of LDL cholesterol and preventing cardiovascular disease. Pravastatin is a lipo-protein lowering drug. The drug works by inhibiting the function of HMG-CoA reductase by occupying the site of the enzyme. This process takes place primarily in the liver, thus slowing the production of cholesterol in the body. Atherosclerosis, or hardening of the arteries, occurs when fat, cholesterol, and other substances build up in the walls of arteries and form plaques. This causes a decrease in blood flow, thereby the O_2 supply to the heart, brain, and other parts of the body. Lowering blood levels of cholesterol and other fats may help to decrease an individual's chances of receiving a myocardial infarction. Pravastatin also works to enhance the antioxidant defense system and inhibit apoptosis, which along with possible improvements in blood circulation make pravastatin a potential therapeutic agent to protect against NIHL.

CHAPTER 2

Materials and Methods

A total of 6 male and 6 female Fischer 344/NHsd rats were used in the study. They were obtained from Harlan Laboratories at age 2 – 3 months. The animals were housed in The Ohio State University Laboratory Animal Resources colony adjacent to the laboratory in which the experiment was conducted. The animals were divided into 1 experimental group and 1 control group (3 males and 3 females in each group). The noise levels did not exceed 60 dB (L_{eq}) over any 24-hour period in the housing facility. Ambient sound levels were monitored with a sound level meter (Larson Davis LxT1 and ACO ½ inch condenser microphone). All procedures involving use and care of the animals were reviewed and approved by The Ohio State University Institutional Animal Care and Use Committee.

2.1. Pravastatin treatment

Pravastatin was dissolved in distilled water at a concentration of 1.56 mg/ml. To investigate protective effects, 4 injections were administered: 24 hours before noise exposure; 1 hour prior to and 1 hour following noise exposure; and then 24 hours post noise exposure. For each animal, a dose of 12 mg/kg was delivered in each intraperitoneal injection, for a total volume of ~2 ml per injection. Equivalent volumes of distilled water were administered to the control group.

2.2. Evoked potential testing

Hearing thresholds were obtained by recording free-field auditory brainstem response (ABR). For all ABR test procedures, the animals were anesthetized with inhalant isoflurane (4% for induction, 1.5% for maintenance, 1 L/min O_2 flow rate). Needle recording electrodes were placed at the vertex (non-inverting), below the left pinna (inverting), and below the right pinna (ground). For threshold testing, test stimuli consisted of alternating phase tone bursts at frequencies 5, 10, 15, 20, 30, and 40 kHz. All stimuli were generated using Tucker Davis Technologies (TDT, Gainesville, FL) SigGen software. Each tone burst was 1 ms in duration, and had a 0.5 ms rise/fall time with no plateau. Each tone burst was gated through a Blackmann window, and presented at a rate of 21/sec. Signals were routed to a speaker (TDT Model MF1) positioned at zero degrees azimuth, 17 cm from the vertex of each rat's head. Acoustic stimuli were calibrated prior to each testing session by recording the output of the speaker with a microphone placed at the animals' head level. The rats' evoked responses were amplified with a gain of 50,000, using a TDT RA4LI headstage connected to an RA4PA pre-amplifier, and bandpass filtered from 100 – 3000 Hz. 250 sweeps were averaged at each stimulus level using TDT BioSig software. The level of the signal was decreased in 5 dB steps from 90 dB SPL until threshold was reached. Threshold was defined as the lowest level at which a detectable response was elicited.

2.3. Noise exposure

Each animal was exposed to a noise exposure condition. The noise was a 2-octave band continuous noise of 4 kHz – 16 kHz, delivered at 110 dB SPL combined with 120 dB pSPL impacts. The rate of impacts was 1/sec. The duration of the combined

continuous and impact noise was 120 minutes. The noise was created on TDT RPvdsEX visual design software and then generated using a TDT RP2 Real time signal processor, amplified by a Marathon DJ-5000 power amplifier (New York, NY). The noise signal was then delivered to a speaker (Vifa D25AG35 1" Dome Tweeter, Madisound Speaker Components, Inc., Middleton, WI) mounted on the side of a wire cage in which the animals were held for the noise exposure. The noise level was calibrated at the level of the animals' head utilizing a LxT1 sound level meter (Larson Davis Inc., Depew, New York) and a 1/2'' condenser microphone. Food and water were available throughout the duration of the noise exposures.

2.4. Assessment of threshold shift

Threshold shifts were calculated by subtracting pre-exposure thresholds from those obtained 24 hours and 14 days post noise exposure to investigate TTS and 28 days post noise exposure to investigate PTS.

2.5. Statistical analysis

A 2-factor ANOVA (Group x Frequency) was used to test pre-exposure thresholds between the 2 groups, and a 3-factor ANOVA (Group x Frequency x Day) was used to analyze differences between the mean ABR thresholds of the 2 groups across the 6 different test frequencies (5, 10, 15, 20, 30, and 40 kHz) pre- and post-noise exposure.

CHAPTER 3

RESULTS

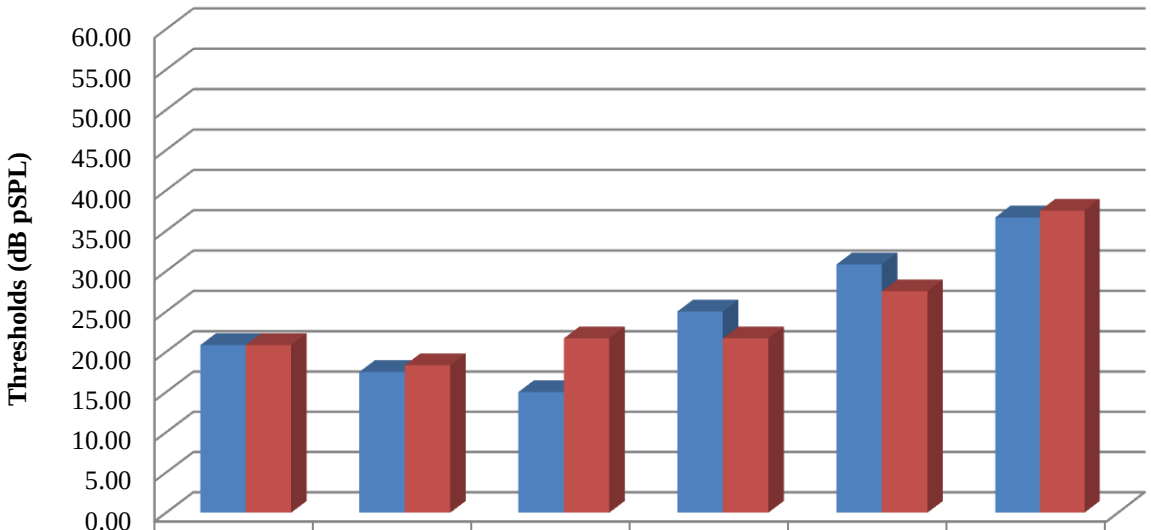
Mean pre-noise exposure thresholds are displayed in Figure 1. The 2-way ANOVA did not show a significant main effect ($p < .05$) of group, but did reveal a significant main effect of frequency ($p < 0.01$). The main effect of frequency was expected due to the higher mean thresholds at 30 kHz and 40 kHz, compared with the lower frequencies 5 kHz – 15 kHz. The lack of effect of group indicated that the rats in the pravastatin group had hearing that was equally sensitive as the rats in the control group.

Threshold shifts for the 2 groups at the 6 frequencies are shown for Day 1 (Figure 2) and Day 14 (Figure 3) to document TTS and Day 28 (Figure 4) to document PTS. Generally, across the frequencies, the 3-way ANOVA also did not show a significant main effect ($p < .05$) of Group, Frequency, and Day, nor any significant interaction involving groups. There was a trend toward a significant interaction of Day and Group ($p = 0.057$), suggesting that with larger sample sizes, a significant interaction might have been found. From Day 1 – 14, a threshold shift recovery was documented. From Day 14 – 28, a flat HL was skewed across the low frequencies, and then an increase in thresholds was noted at 30 kHz and 40 kHz, indicating threshold shift recovery.

For further clarification of the data, plots were created that show recovery functions from Day 1 to Day 28 at each of the tested frequencies: 5 kHz (Figure 5), 10

kHz (Figure 6), 15 kHz (Figure 7), 20 kHz (Figure 8), 30 kHz (Figure 9), and 40 kHz (Figure 10). Largely, the recovery functions indicate that, from Day 14 to Day 28, the treated group demonstrated a decrease in thresholds while the untreated group's thresholds actually increased.

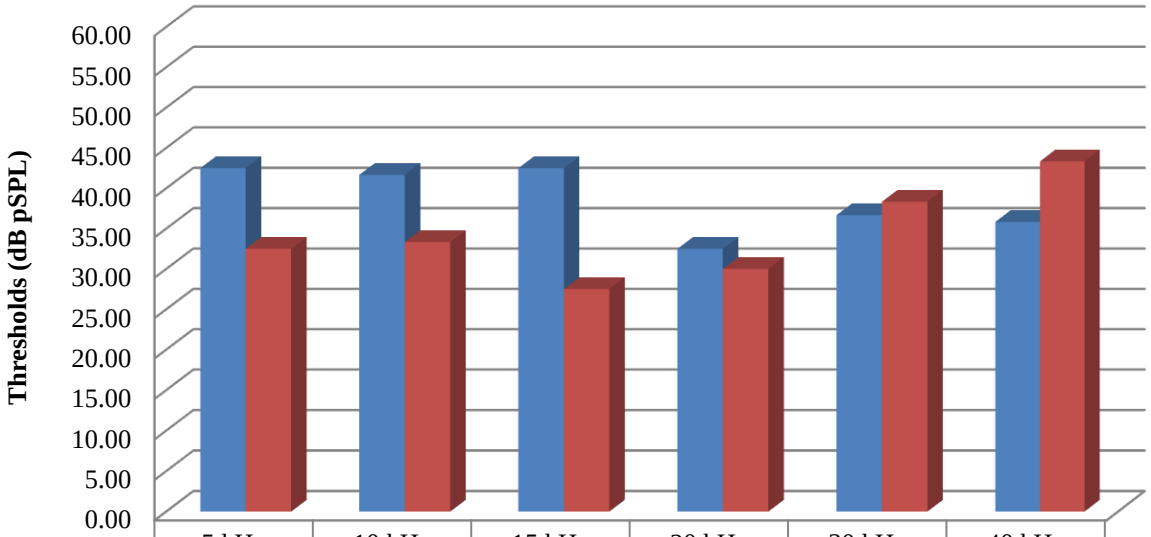
Figure 1
Pre-Noise Exposure Thresholds



	5 kHz	10 kHz	15 kHz	20 kHz	30 kHz	40 kHz
Treated	20.83	17.50	15.00	25.00	30.83	36.67
Untreated	20.83	18.33	21.67	21.67	27.50	37.50

Figure 1. Mean pre-noise exposure thresholds for the treated and untreated groups.

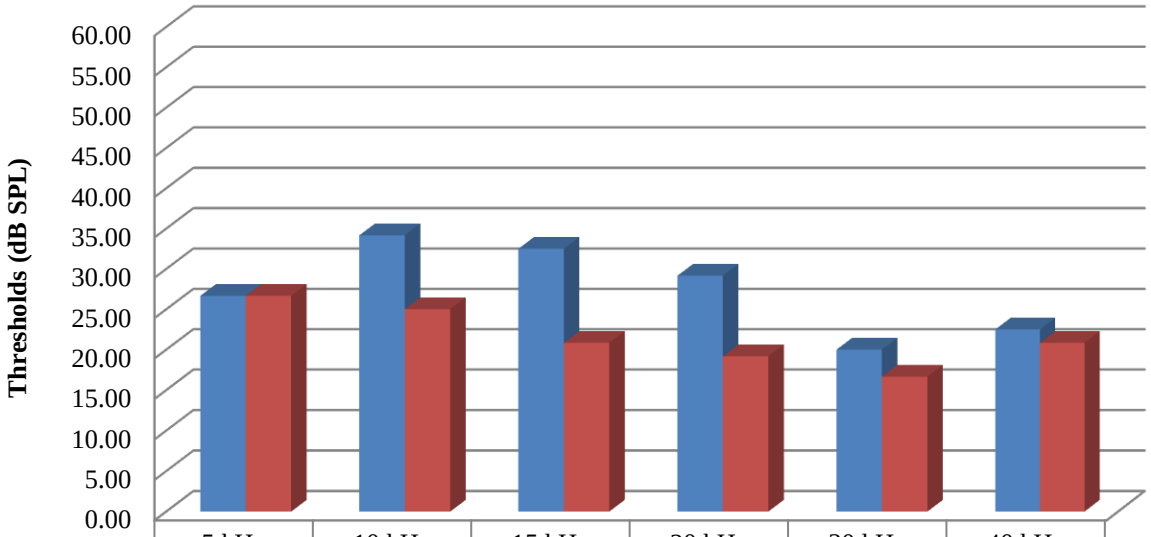
Figure 2
Day 1: Noise Exposure Threshold Shift



	5 kHz	10 kHz	15 kHz	20 kHz	30 kHz	40 kHz
Treated	42.50	41.67	42.50	32.50	36.67	35.83
Untreated	32.50	33.33	27.50	30.00	38.33	43.33

Figure 2. Day 1 noise exposure threshold shift for the treated and untreated groups.

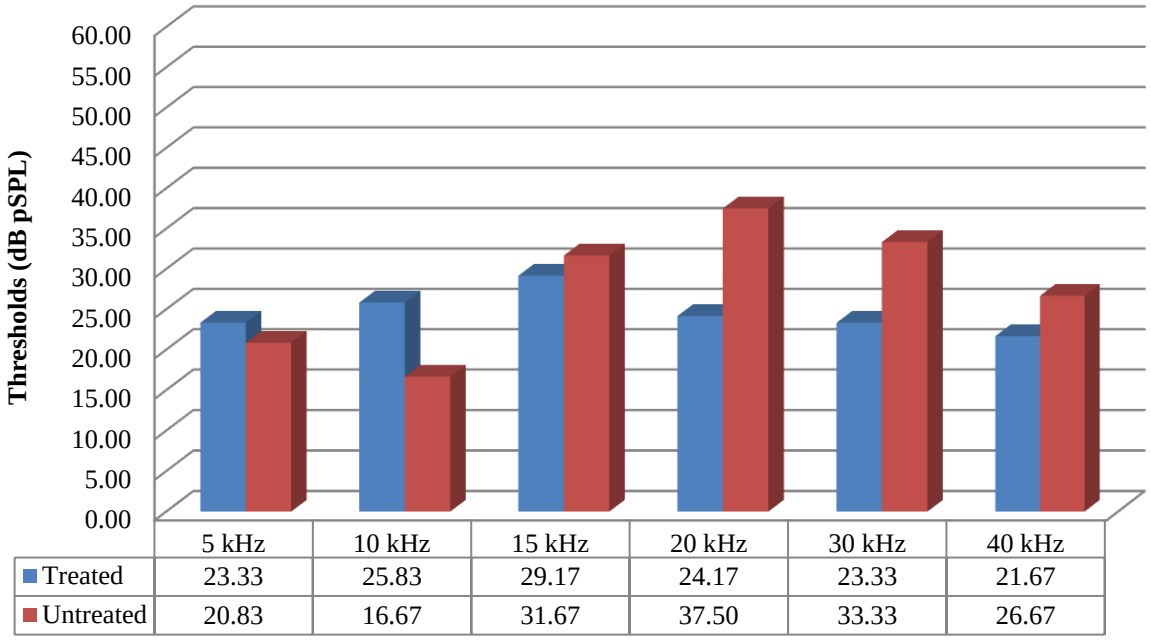
Figure 3
Day 14: Noise Exposure Threshold Shift



	5 kHz	10 kHz	15 kHz	20 kHz	30 kHz	40 kHz
Treated	26.67	34.17	32.50	29.17	20.00	22.50
Untreated	26.67	25.00	20.83	19.17	16.67	20.83

Figure 3. Day 14 noise exposure threshold shift for the treated and untreated groups.

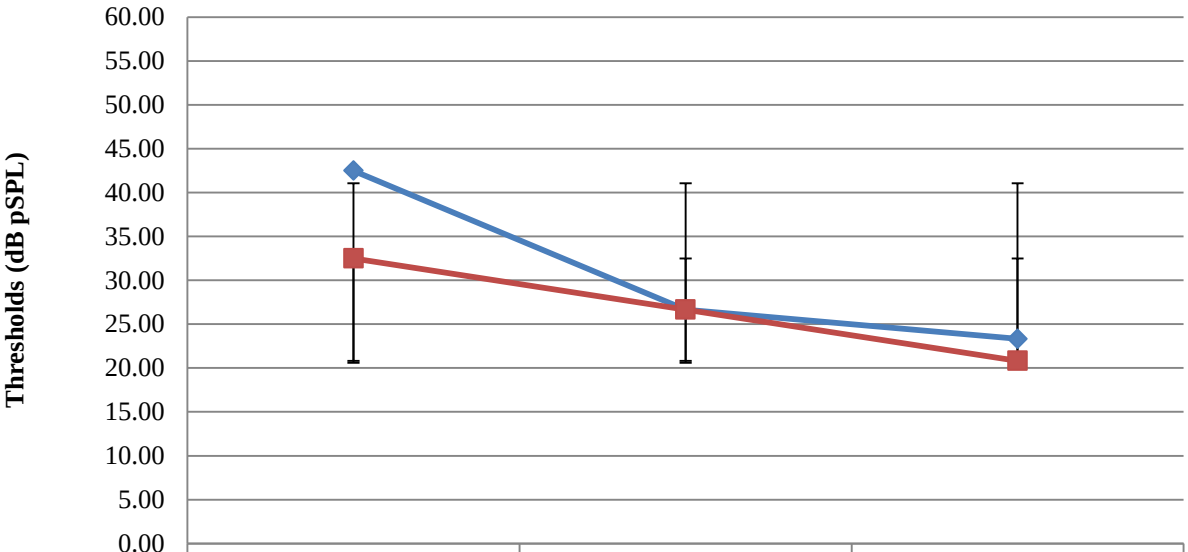
Figure 4
Day 28: Noise Exposure Threshold Shift



	5 kHz	10 kHz	15 kHz	20 kHz	30 kHz	40 kHz
Treated	23.33	25.83	29.17	24.17	23.33	21.67
Untreated	20.83	16.67	31.67	37.50	33.33	26.67

Figure 4. Day 28 noise exposure threshold shift for the treated and untreated groups.

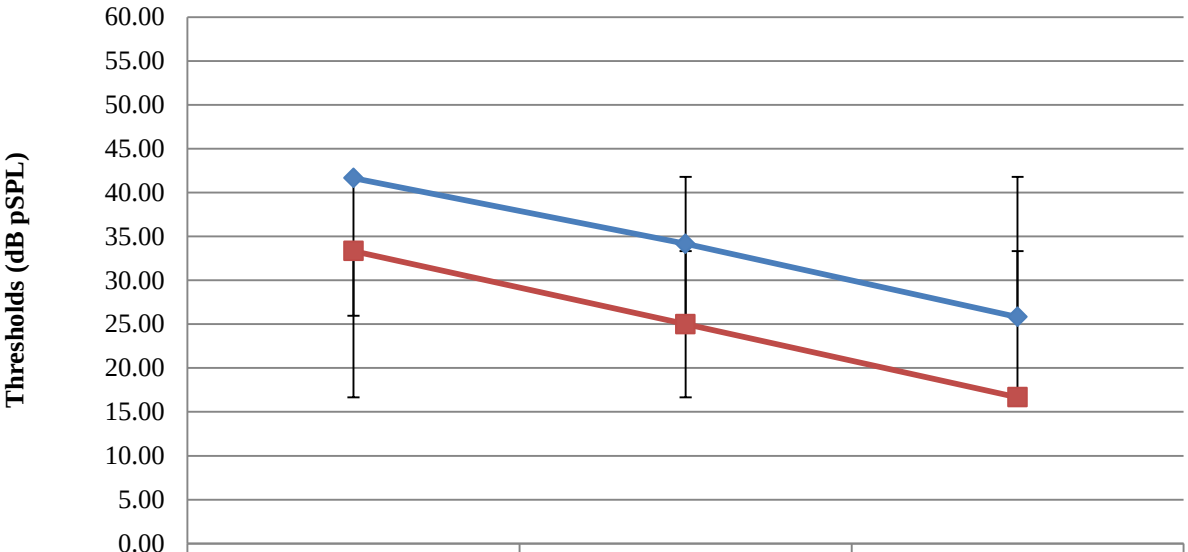
Figure 5
5 kHz: Treated Group vs. Untreated Group Across Days



	Day 1	Day 14	Day 28
Treated	42.50	26.67	23.33
Untreated	32.50	26.67	20.83

Figure 5. Day 1 - Day 28 threshold recovery function (5 kHz) for the treated and untreated groups.

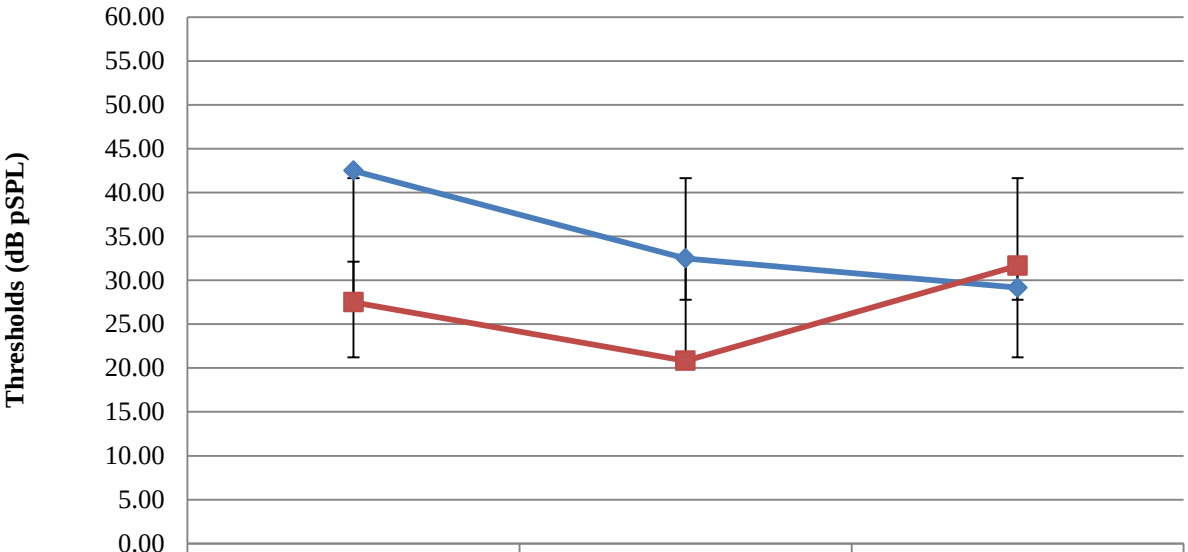
Figure 6
10 kHz: Treated Group vs. Untreated Group Across Days



	Day 1	Day 14	Day 28
Treated	41.67	34.17	25.83
Untreated	33.33	25.00	16.67

Figure 6. Day 1 - Day 28 threshold recovery function (10 kHz) for the treated and untreated groups.

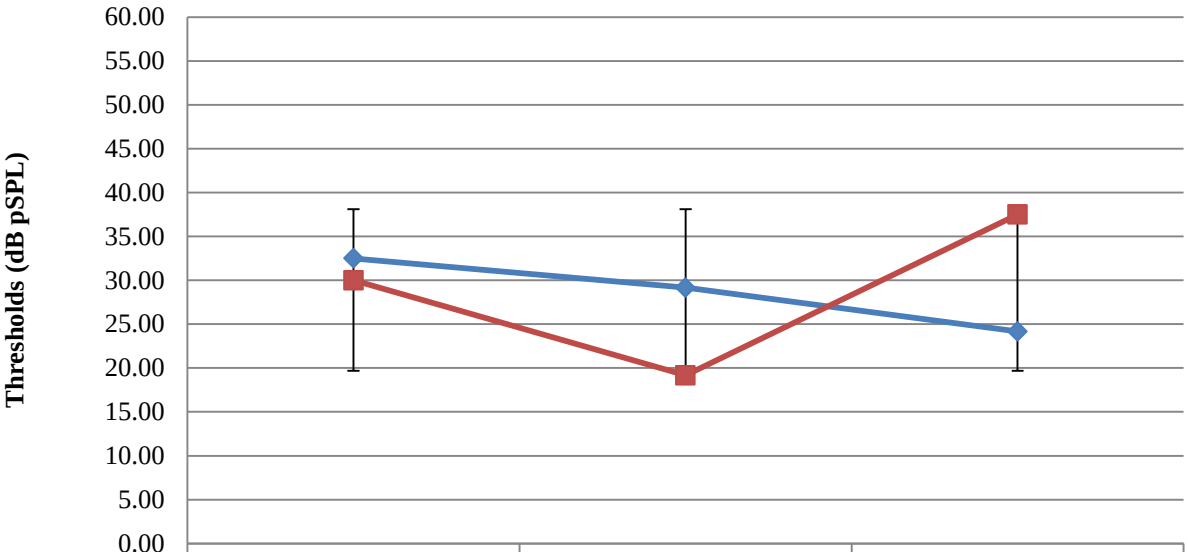
Figure 7
15 kHz: Treated Group vs. Untreated Group Across Days



	Day 1	Day 14	Day 28
Treated	42.50	32.50	29.17
Untreated	27.50	20.83	31.67

Figure 7. Day 1 - Day 28 threshold recovery function (15 kHz) for the treated and untreated groups.

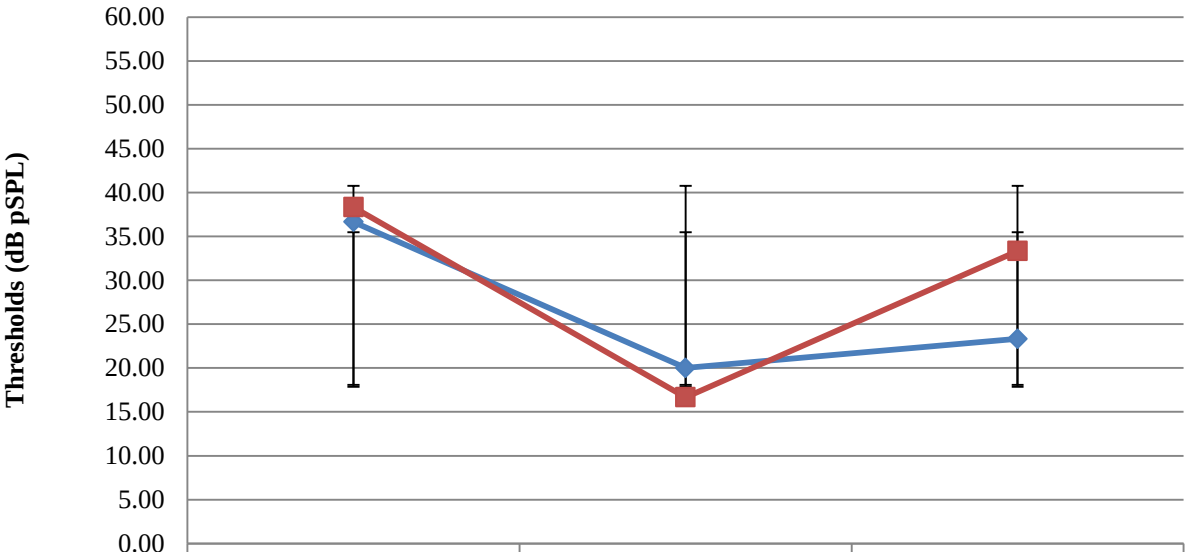
Figure 8
20 kHz: Treated Group vs. Untreated Group Across Days



	Day 1	Day 14	Day 28
Treated	32.50	29.17	24.17
Untreated	30.00	19.17	37.50

Figure 8. Day 1 - Day 28 threshold recovery function (20 kHz) for the treated and untreated groups.

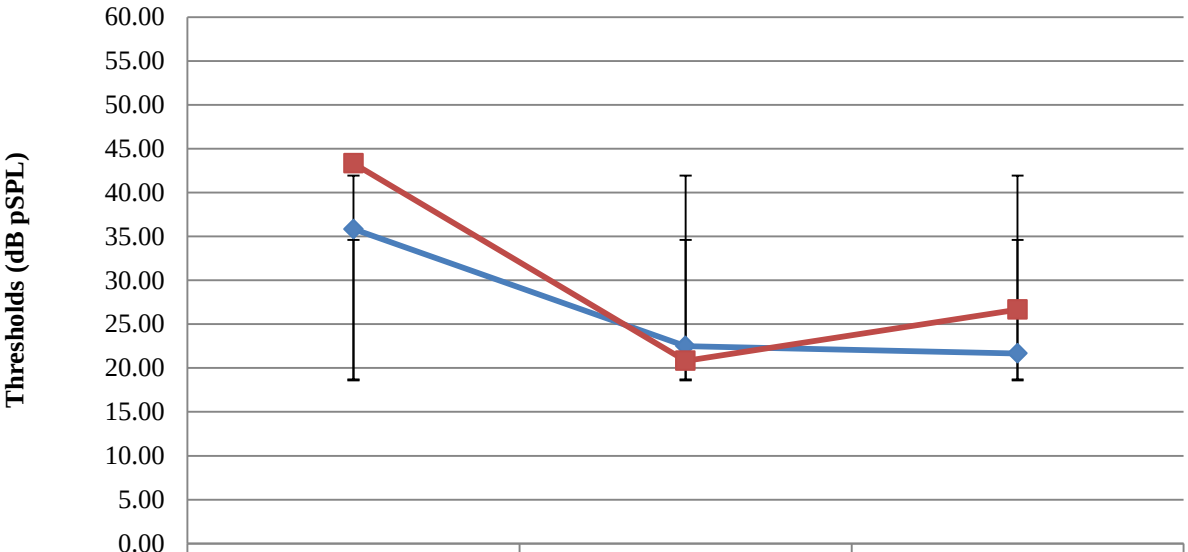
Figure 9
30 kHz: Treated Group vs. Untreated Group Across Days



	Day 1	Day 14	Day 28
Treated	36.67	20.00	23.33
Untreated	38.33	16.67	33.33

Figure 9. Day 1 - Day 28 threshold recovery function (30 kHz) for the treated and untreated groups.

Figure 10
40 kHz: Treated Group vs. Untreated Group Across Days



	Day 1	Day 14	Day 28
Treated	35.83	22.50	21.67
Untreated	43.33	20.83	26.67

Figure 10. Day 1 - Day 28 threshold recovery function (40 kHz) for the treated and untreated groups.

CHAPTER 4

Discussion

The role of pharmacological agents in the management of NIHL continues to be expanded. This study did not show a statistically significant protective effect of pravastatin, a rate-limiting enzyme of cholesterol synthesis, in Fischer 344/NHsd rats. Since the time this study was conducted, pravastatin has been shown to have a protective effect against cochlear injury in mice. In a study administered by Park et al. (2012), pravastatin reduced HC death in the cochlea after noise exposure and decreased threshold shifts as well. Furthermore, noise-induced increases in 4-HNE, which is a byproduct of lipid peroxidation in the cell membrane, were found to be reduced by pravastatin treatment. Additionally, Rac1, a subunit of the NADPH oxidase complex, was inhibited by the administration of pravastatin. The NADPH oxidase complex is a superoxide generator. The study suggests pravastatin may protect the cochlea against NIHL by lowering ROS overproduction.

What remains unclear is why the current study was unsuccessful in protecting against NIHL. The possibility exists that the sample sizes of 6 rats per group was too low to see a significant effect, since there was a statistical trend for a Group x Day interaction. That interaction is not clearly showing protection, since the control group showed lower mean threshold shifts at Day 14 than the treated group. Though, on Day 28, the treated

animals had lower threshold shifts than the controls. Expanding the study to include more animals would likely help make the nature of an interactive effect clearer.

Also, in the Park et al. (2012) study, BALB/c mice were utilized, which may have contributed to the positive outcome of the study. Mice animal models are the predominant model for hearing research, primarily with regards to age-related HL (ARHL) and NIHL studies (Ohlemiller, 2006). There are numerous reasons that have contributed to the growth in the application of mice in auditory research. The effects of noise in mouse cochleae have been studied more extensively than in the Fischer 344/NHsd rat. Mouse studies have revealed peripheral pathology in addition to transneuronal effect of noise trauma. Practical advantages of utilizing mice include their short life span; therefore, the effects of noise (and age) are apparent in a shorter timeframe. According to Zheng, Johnson, and Erway (1999), many inbred strains of mice exhibit a degree of delayed, progressive hearing loss, which is beneficial in reviewing mice model findings and integrating such findings within the contextual basis of NIHL.

Additionally, there is an ease of genetic standardization and genetic engineering (Ohlemiller, 2006). The mouse genome can be applied to humans since mice have a genetic similarity to humans in addition to minimal variance within strains. Mice are vulnerable to noise; in fact, across inbred strains, there is widespread discrepancy in noise vulnerability, which can be particularly useful for studies of NIHL. There are genetic influences on noise susceptibility. Mouse genes and alleles may promote NIHL (Ohlemiller, 2006). To demonstrate, B6 mice follow autosomal recessive inheritance (Erway, Shiau, Davis, & Kreig, 1996). These mice demonstrate a progressive loss of HC, with a degeneration of afferent neurons, stria vascularis, and spiral ligament.

Mouse cochleae are short in length, thus allowing for fewer basal-apical sampling points in studies designed to quantitatively assess HC and neuronal counts in the organ of Corti, spiral ligament and spiral limbus, and the stria vascularis in addition to classification of afferent synapses (Ohlemiller, 2006). Acoustic injury to 1 cell type may aggravate injury to other cell types. It is important to note that it may be challenging to provide a direct comparison between mice and human hearing sensitivity, in terms of determining which cochlear regions are adversely affected, since mouse and human hearing overlap over roughly 3 octaves (~2.5 kHz – 20 kHz) (Ohlemiller, 2006).

Additionally, in certain strains of mice, the major form of the melanin pigment, eumalanin, may protect the cochlear lateral wall by providing antioxidant effects (Barrenas, 1997). Oxidative stress may be exacerbated by the melanin isoform, pheomelanin. Eumalanin has a role in protecting cochlear HC from noise trauma. Moreover, minimal reduction in endocochlear potential (EP), as well as corresponding anatomical correlates of EP, was demonstrated post noise exposure.

There is an interactive effect between age and noise. Mice models provide evidence of windows of increased susceptibility to noise exposure. A period of heightened vulnerability is from adolescence into early adulthood, which is up to approximately 4 months of age in mice. In the Park et al. (2012) study, the mice were 7 – 8 weeks old. In the current study, the rats were 2 – 3 months old. This suggests that the age of the animals should not have contributed to the susceptibility of the noise exposure. Overall, mouse models may be more susceptible to NIHL. If a group of animals is more susceptible to noise trauma, then pravastatin may provide a greater protective effect against NIHL in those animals.

Different doses and varying time schedules of administration need to be tested in subsequent studies to determine whether a statistically significant protective benefit of pravastatin against NIHL can be achieved in rat animal models. To illustrate, in the study administered by Park et al. (2012), the BALB/c mice received a pre-treatment with pravastatin (25 mg/kg) for 5 days prior to noise exposure. In the current study, 4 injections were administered (12 mg/kg) in Fischer 344/NHsd rats: 24 hours before noise exposure; 1 hour prior to and 1 hour following noise exposure; and then 24 hours post exposure. Therefore, the total amount of pravastatin delivered in the Park et al. (2012) study was substantially greater than the amount given in the current study. Pre-treating with higher doses over a long period of time before the noise could easily create a more successful protection effect.

To further explain the discrepancy between the current study and the Park et al., (2012) study, one must consider the type of noise. In the Park et al. (2012) study, animals were exposed to a 112 dB SPL broadband white noise (1 kHz – 20 kHz) for 3 hours. In the current study, as previously mentioned, the noise was a 2-octave band continuous noise of 4 kHz – 16 kHz. The noise was delivered at 110 dB SPL combined with 120 dB pSPL impacts, and the rate of impacts was 1 per second. The duration of the combined continuous and impact noise was 120 minutes. A chief difference between the current study and the study conducted by Park et al. (2012) is the utilization of impact noise. Noise damage negatively affects cellular subsystems of the IE. Impact sounds, such as gunfire, are particularly menacing to the cochlea since it may lead to immediate mechanical damage that cannot be prevented pharmacologically. The impact noise in this study may have caused greater damage than a cochlea not otherwise exposed to impact

noise. This suggests that the impact noise may have prevented the pravastatin from providing optimal protective assistance.

Furthermore, the mice in the Park et al. (2012) study were examined immediately and within 24 hours post noise exposure in addition to 14 days after noise exposure to evaluate PTS. In this study, the rats were examined 1 hour before, 1 hour after, and 24 hours post noise exposure, similar to the Park et al. (2012) study. However, the rats were evaluated 28 days post noise exposure to evaluate PTS in this study. Evaluating the animals over a greater time period will more than likely be valuable in determining over what days, if at all, the greatest amount of threshold shift recovery occurs.

In addition to the type of noise used, researchers much consider how the method of administering the drug may affect the protective capacity of pravastatin. In the current study, a dose of 12 mg/kg was delivered in each intraperitoneal injection. In the Park et al. (2012) study, the mice were pre-treated with 25 mg/kg of pravastatin orally via gavage. Because of this method of administration, and since a greater dosage of pravastatin was utilized, Park and his colleagues were possibly able to introduce more drug into the cochleae. However, because the exact amount of the drug that reaches the cochleae is not known, it is difficult to regulate dosages and time schedules of administration.

The potential mechanisms of action of pravastatin in the cochlea are still unclear. The relationship between cholesterol and auditory function has been previously reviewed. According to Levic and Yamoah (2011), HC development and OHC tuning is in part due to membrane cholesterol. However, high triglyceride levels and plasma cholesterol may augment the development of HL due to acoustic overstimulation. This may be due to

increased blood viscosity and atherosclerosis (Sutbas et al., 2007). Moreover, HC may demonstrate a larger uptake of cholesterol due to an increased stiffness in the IE, thus promoting a greater degree of hearing loss. Statins are thought to lessen the inflammatory responses of the IE as well as provide vascular protective effects by regulating ROS levels (Ohlemiller, 2006). Ischemia injury from the stria vascularis is an underlying mechanism for NIHL, and as such statins affect the stria vascularis, thereby attenuating the effects of NIHL via the inhibition of the NADPH oxidase complex formation (Park et al., 2012).

CHAPTER 5

Conclusion

NIHL is a leading occupational disease and contributes to the development and progression of ARHL (Lynch & Kil, 2005). It is known that acoustic trauma can lead to a physical disruption of the organ of Corti along with necrosis and apoptosis at the molecular level. Consequently, for the past 20 years, the pharmacological prevention and treatment of NIHL has been investigated (Lynch & Kil, 2005). In humans, a lack of noise-related cochlear injury studies have been observed due to incomplete noise histories and since most temporal bones examined demonstrate mixed pathology (Ohlemiller, 2006). Randomized, double-blinded, placebo controlled studies in humans is needed to investigate the effects of pravastatin in humans, eventually leading to the development of clinical application. Drugs that successfully prevent or treat NIHL will more than likely have a substantial impact on one's overall quality of life, including medical costs and disability compensation (Lynch & Kil, 2005). Currently, otoprotective agents have not received FDA approval for clinical use. In the foreseeable future, audiologists will be working with patients and physicians, either for clinical applications or research purposes, in selecting and monitoring pharmacological otoprotective agents against NIHL.

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